UNIVERSITI PUTRA MALAYSIA

INFECTIOUS BURSAL DISEASE VIRUS: ITS GENOMIC PROPERTIES, EVOLUTION, AND INFECTION TO THE HEAD-ASSOCIATED LYMPHOID TISSUES OF CHICKEN

TAN DO YEW

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By

TAN DO YEW

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2004

DEDICATION

To my parents, grandma, and siblings.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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September 2004

Chairman: **Associate Professor Mohd Hair Bejo, Ph.D.**

Faculty: **Veterinary Medicine**

Infectious bursal disease (IBD) is an important immunosuppressive viral disease of chicken caused by IBD virus (IBDV). There are several strains of IBDV, and vaccination may not protect the chicken against all strains. Therefore as for control and prevention effort, it is important to know which strain is present in the field. In this study, four IBDV isolated from Malaysia had been characterized in detail. The isolates were B00/73, B00/81, 94230, and 94268. Based on their pathogenicity and sequence characteristics, these isolates were identified as very virulent strain of IBDV (vvIBDV). Further analyses of the genetic sequences of 131 IBDV isolates had provided new insights into the genomic properties of IBDV. These include its bias in base usage, the avoidance of CpG and TpA dinucleotides, the unique dinucleotide

pattern of vvIBDV, and its acquisition of VP5 gene using overprinting strategy. Meanwhile, a better approach in studying the molecular evolution of IBDV was introduced and termed as “holistic approach of phylogenetic analysis”. The approach widens the perspective of phylogenetic analysis and reduces error in phylogenetic inferences. Using this approach, IBDV isolated from Malaysia were shown to share a common origin with other foreign vvIBDV isolates. In addition, the vvIBDV isolated from village chicken (94268 isolate) was found to be evolutionary closely related to the isolates that affected the commercial chickens; indicating infection by vvIBDV is not limited within the farm boundary. This study also inquired into the cellular response of head-associated lymphoid tissues (HALT) following intraocular infection of vvIBDV. The two major lymphoid tissues of HALT are Harderian gland and conjunctiva-associated lymphoid tissue; in which together, both are indispensable for the local immunity in the paraocular region. HALT was impaired by vvIBDV infection at day 3-4 post-inoculation, but swiftly recovered at day 10. The destruction of HALT, though transient, may interfere with the vaccination programme for other respiratory diseases and give chance to opportunistic infection to the ocular and respiratory tract of chicken.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

VIRUS PENYAKIT BURSA BERJANGKIT:
SIFAT-SIFAT GENOMIK, EVOLUSI, DAN JANGKITANNYA KEPADA TISU LIMFOID BERSEKUTU KEPALA AYAM

Oleh

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Penyakit bursa berjangkit (IBD) ialah sejenis penyakit ayam yang amat penting di mana ia boleh melumpuhkan sistem imun ayam dan diakibatkan oleh virus IBD (IBDV). Terdapat beberapa jenis strain IBDV yang berlainan. Vaksinasi terhadap satu strain tidak semestinya boleh melindungi ayam daripada ancaman strain yang lain. Oleh yang demikian, adalah mustahak untuk mengetahui strain IBDV yang wujud di ladang penternakan untuk kawalan dan pencegahan IBD. Dalam kajian ini, empat IBD yang diasingkan dari Malaysia telah dicirikan secara terperinci. Virus ini ialah B00/73, B00/81, 94230, dan 94268. Dengan merujukkan kepada keupayaan virus untuk mengakibatkan penyakit IBD dan jujukan genetiknya, virus ini telah dikenalpasti sebagai strain amat virulen IBDV (vvIBDV). Penganalisisan selanjutnya

yang menggunakan 131 jujukan genetik telah memberi gambaran baru terhadap sifat genomik IBDV. Sifat ini termasuk kecenderungan virus dalam penggunaan nukleotid tertentu, penghindaran pasangan nukleotid CpG dan TpA, corak unik pasangan nukleotid vvIBDV, dan pemerolehan gen VP5 dengan menggunakan strategi pertindihan. Sementara itu, pendekatan kajian evolusi molekul yang lebih baik juga diperkenalkan dan dinamakan sebagai "Pendekatan keseluruhan dalam analisis filogenetik". Pendekatan baru ini dapat meluaskan perspektif analisis filogenetik dan mengurangkan kesilapan dalam membuat kesimpulan filogenetik. Dengan menggunakan pendekatan keseluruhan, IBDV yang diasingkan di Malaysia telah ditunjukkan berasal dari punca yang sama dengan virus IBD negara asing. Di samping itu, vvIBDV yang diasingkan daripada ayam kampung (virus 94268) mempunyai hubungan evolusi yang rapat dengan virus yang menjangkit ayam komersil. Ini menunjukkan jangkitan vvIBDV adalah tidak terbatas kepada ladang sahaja. Kajian ini juga menyiasat gerak balas tisu limfoid bersekutu di kepala (HALT) ayam berikutan jangkitan intraokulus oleh vvIBDV. HALT terdiri daripada dua tisu limfoid yang utama, iaitu kelenjar Harderian dan tisu limfoid bersekutu di konjunktiva. Kedua-duanya adalah amat diperlukan untuk pelalian setempat di persekitaran mata. Jangkitan vvIBDV merosakkan HALT pada hari yang ketiga dan keempat sesudah inokulasi virus. Akan tetapi, HALT pulih dengan cepat pada hari yang kesepuluh. Pemusnahan HALT, meskipun sementara, mungkin boleh menjejaskan program vaksinasi terhadap penyakit-penyakit salur pernafasan.

Tambahan lagi, ini akan memberi kesempatan kepada penyakit-penyakit lain untuk menjangkit kawasan sekitar okulus dan saluran pernafasan ayam.

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I certify that an Examination Committee met on 13th September 2004 to conduct the final examination of Tan Do Yew on his Doctor of Philosophy thesis entitled "Infectious bursal disease virus: its genomic properties, evolution, and infection to the head-associated lymphoid tissues of chicken" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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TAN DO YEW

Date:

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LIST OF ABBREVIATIONS

A	Adenine
AA	Amino acid sequences
AGID	Agar gel immunodiffusion
AGPT	Agar gel precipitation test
atIBDV	Attenuated strain of infectious bursal disease virus
ayIBDV	Atypical strain of infectious bursal disease virus
BF	Bursa of Fabricius
BI	Bursal index
BP	Bootstrap percentage/bootstrap percentages
BSA	Bovine serum albumin
BSNV	Blotched snakehead virus
C	Cytosine
CALT	Conjunctiva-associated lymphoid tissue
CAM	Chorioallantoic membrane
CAV	Chicken anaemia virus
cDNA	Complementary deoxyribonucleic acid
CDS	Coding sequence
CEF	Chicken embryo fibroblast
CMI	Cell-mediated immunity

cvIBDV	Classical strain of infectious bursal disease virus
d	Day
DAB	Diaminobenzidine tetrahydrochloride
ddH_2O	Deionized double-distilled water
DEPC	Diethyl pyrocarbonate
DF-PCA	Principal component analysis of dinucleotide frequencies
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DnI	Dinucleotide index
dNTP	Deoxynucleoside triphosphate
dsDNA	Double-stranded DNA
DTT	Dithiothreitol
DXV	Drosophila X virus
ELD_{50}	Fifty percent embryo lethal dose
ELISA	Enzyme-linked immunosorbent assay
G	Guanine
h	Hour
HALT	Head-associated lymphoid tissues
HE	Haematoxylin-and-eosin
HG	Harderian gland
HVR	Hypervariable region

IB	Infectious bronchitis
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus/Infectious bursal disease viruses
IBDV-BDA	IBDV-bursal disease antibody
IBV	Infectious bronchitis virus
ICX	IBDV immune complex vaccine
IPNV	Infectious pancreatic necrosis virus
IPS	Immunoperoxidase staining technique
JC69	Jukes and Cantor 1969 model
JTT	Jones-Taylor-Thornton model
K2P	Kimura's two-parameter model
LB	Luria-Bertani
LE	Lymphoepithelium
ML	Maximum likelihood
MP	Maximum parsimony
ND	Newcastle disease
NDV	Newcastle disease virus
NT	Nucleotide sequences
OIE	Office international des epizooties
ORF	Open reading frame
PBS	Phosphate-buffered saline

PC	Plasma cell/plasma cells
PCR	Polymerase chain reaction
PI	Post-inoculation
RBC	Red blood cells
RE	Restriction enzyme
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SDS	Sodium dodecyl sulfate
SPF	Specific-pathogen-free
SPIEM	Solid phase immune electron microscopy
T	Thymine
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
TNE	Tris-natrium-EDTA
TEM	Transmission electron microscopy
UPGMA	Unweighted pair-group average
vaIBDV	Variant strain of infectious bursal disease virus
VLP	Virus-like particles
VN	Virus neutralization
VNF	Virus neutralization factor
vvIBDV	Very virulent strain of infectious bursal disease virus

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CHAPTER 1

INTRODUCTION

Infectious bursal disease (IBD) is defined, by the Office International des Epizooties (OIE), as a highly contagious viral disease that destructs the lymphoid organs of young chickens specifically the bursa of Fabricius, and leads to immunosuppression and death. IBD topped the list of most serious poultry diseases in a survey conducted by *World Poultry* in 1999 (van der Sluis 1999), testifying its pressing threat to the poultry industry worldwide.

IBD is also known as “Gumboro disease” because it was first reported in Gumboro, Delaware (USA) by Cosgrove in 1962. Nonetheless, to accurately reflect the characteristics of the disease, it was later termed “infectious bursal disease” by the typical lesions found in the infected bursa of Fabricius. The causative agent of IBD is IBD virus (IBDV), a double stranded RNA (dsRNA) virus in the genus of *Avibirnavirus* (Dobos and others 1979). IBDV targets and destructs actively dividing immature B-lymphocytes (Burkhardt and Muller 1987), in which these cells are important for the chicken immune system. Therefore, the infected birds are immunosuppressed because their immune system had been decimated by IBDV.